ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149321 Year: 98 Project Number: 1265-31000-061-00 D
Mode Code: 1265-45-00 STP Codes: 3.1.2.1 100%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

Title: GENETIC MAPPING OF QUANTITATIVE TRAIT LOCI IN DAIRY CATTLE

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

This project focuses on the identification of DNA markers associated with milk production, protein and fat yields, protein and fat percentages, productive life, somatic cell score (an indirect measure of mastitis), calving ease, and conformation traits in dairy cattle. Although selection to improve milk production has been effective using traditional methods, the rate of improvement could be increased using marker assisted selection. Furthermore, traditional methods of selection have difficulty improving lowly heritable traits, such as reproduction and disease resistance. Application of marker-assisted selection for milk production and other economically important traits, such as disease resistance, reproduction, length of productive life, and conformation traits associated with fitness, would give US dairymen a tremendous increase in the rate of genetic improvement on these traits while accelerating genetic improvement for production. Prior to implementation of marker-assisted selection, either DNA markers or causative genetic variation associated with these important traits must be identified so that current generations of genetically superior animals having unique combinations of traits can be distinguished.

2. How serious is the problem? Why does it matter?

It is estimated that mastitis costs dairy producers $2 billion, milk fever $216 million, and ketosis $48 million each year. These costs have a huge impact on the sustainability of farmers, dairy processors, and supporting industries, and these costs are increasing each year. Research has shown that selection on fertility needs to be practiced

since fertility decreases in a herd as milk production increases, and culling due to infertility is the primary cause of involuntary culling in dairy herds. In addition, reducing mastitis incidence genetically would reduce antibiotic use, resulting in less risk of human exposure to these antibiotics and reducing problems of antibiotic resistant bacteria.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

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National Program 101 Animal Germplasm, Resources, Conservation, and Development (100%)
This research allows scientists to identify DNA markers associated with the genetic variation in genes affecting milk production efficiency, disease resistance, reproductive efficiency, and longevity in dairy cattle. This project also supports the Cooperative Dairy DNA Repository, which is a collection of DNA from well-evaluated bulls as well as bulls with relatively little progeny information. This collection will be used to verify previously identified marker-trait associations and estimate allelic effects across families.

4. What was your most significant accomplishment this past year?

Genetic markers were identified which are associated with a dairy cattle conformation trait that has been attributed to an increased incidence of metabolic disorders such as ketosis and milk fever. The effect linked to this region of the bovine genome appears to have a negligible effect on milk yield traits, thus allowing the selection for reduced disease incidence without affecting potential milk yield.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Over the past three years, seven large US Holstein families were studied to identify DNA markers associated with economically important traits. To date, over 120 DNA markers have been investigated in approximately 950 animals. Phenotypic data from over 20 quantitative traits were used in a statistical analysis to determine which DNA markers were associated with these economically important traits. Seven DNA regions were identified that were associated with highly significant effects on somatic cell score (a measure of mastitis incidence), milk composition, and conformation traits. Additional DNA markers are currently under investigation to more precisely study these important regions of the bovine genome. Information on genetic effects from these studies will allow the dairy cattle breeding industry to improve selection decisions

and increase the health, reproduction, and production of the commercial dairy cow.

In 1998, a Memorandum of Understanding was written to establish the Cooperative Dairy DNA Repository (CDDR). The CDDR will be a collection of DNA from dairy bulls currently undergoing progeny testing in addition to DNA from animals that link these contemporary animals to the 8 families currently under investigation. This project involves participation from the artificial insemination industry through donations of DNA source and scientists from academia, private industry, and the public sector through analysis of the samples.

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6. What do you expect to accomplish during the next year?

During the next year additional markers will be studied in 8 US Holstein families. Emphasis will be placed on DNA regions where a significant association has been identified and on DNA regions where marker coverage is sparse. Markers selected from these respective regions will allow us to confirm the initial findings, refine the genetic intervals containing important traits, and identify additional DNA regions requiring further study.
During 1999 the CDDR is expected to be fully operational through acquisition of additional DNA samples and the establishment of collaborators from academia and private industry. Families will be studied from this collection to provide additional evidence of important DNA regions and estimate allelic effects across families. This research will identify the most favorable alleles in the Holstein population that could be used in a marker assisted selection program.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

No technologies have been transferred to the end user because identification of DNA regions associated with economically important traits is still in its infancy. Our results must be verified in contemporary populations before the information can be transferred to the artificial insemination industry. Confirmation of marker/trait associations will rely on the development of improved statistical analysis of the traits and the refinement of genomic regions containing desired traits with additional polymorphic DNA markers. Without substantial verification, premature commercial application of marker/trait information could lead to financial and genetic losses in addition to the loss of confidence the dairy industry currently places on our research efforts. However, some applicable and

useful genetic information is likely to be available to the industry within the next five years. Constraints on transferring this technology include the need for verification and selection populations, improved statistical methods to detect marker-trait associations, and reliable phenotypic data for reproduction traits.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

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ASHWELL, M.S., DA, Y., VAN TASSELL, C.P., VANRADEN, P.M., et al. 1998. Detection of putative loci affecting milk production and composition, health, and type traits ... population. J. Dairy Sci. 81:3309-3314.
ASHWELL, M.S., DA, Y., VANRADEN, P.M, REXROAD, JR., C.E. and MILLER, R.H. 1998. Detection of potential loci affecting conformational type traits ... using microsatellite markers. J. Dairy Sci. 81: 1120-1125.
VAN TASSELL, C.P., SONSTEGARD, T.S. and ASHWELL, M.S. 1998. Investigation of a quantitative trait locus for dairy form in one family of Holsteins. J. Dairy. Sci. 81(1):73.

PUBLICATIONS:

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ASHWELL, M.S. 1998. Expressed sequence tags isolated from a bovine lactating mammary gland cDNA library. Germplasm Release. Accession Numbers AA908007-AA908030.

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ASHWELL, M.S., DA, Y., VANRADEN, P.M, REXROAD, JR., C.E. and MILLER, R.H. 1998. Detection of potential loci affecting conformational type traits ... using microsatellite markers. J. Dairy Sci. 81:1120-1125.

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ASHWELL, M.S., DA, Y., VANRADEN, P.M., et al. 1998. Detection of putative loci affecting milk production and composition, health, and type traits in a US Holstein population. Plant Anim. Genome 6:155.

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CAPERNA, T.J., SONSTEGARD, T.S. and TALBOT, N.C. 1998. Porcine hepatocytes display either parenchymal or bile duct ... phenotypic and genotypic characterization. J. Ani. Sci. 76(Suppl 1):139.

Publications: (Continued)

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CONNOR, E.E., ASHWELL, M.S., KAPPES, S.M. and DAHL, G.E. 1998. Characterization of a partial bovine cDNA #chromosome 4 using a novel PCR-RFLP. Proc. 3rd Internat. Conf. Farm Anim. Endocrinol. BASE 2:8.

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FREKING, B.A., KEELE, J.W., SONSTEGARD, T.S., et al. 1998. Evaluation of the ovine callipyge locus: I. Relative chromosomal position and gene action. J. Anim. Sci. 76(8):2062-2071.

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KEOWN, J.F., MONTALDO, H., VAN VLECK, L.D. and VAN TASSELL, C.P. 1998. Economic responses and risk from use of selected Holstein sires in ...USA. Proc. 6th World Congr. Genet. Appl. Livest. Prod. 23:327-330.

12.
LOPEZ-CORRALES, N.L., SONSTEGARD, T.S., et al. 1998. Comparative physical mapping of PROC, EN1, ALPI, TNP1, and IL1B genes in cattle and sheep. Proc. European Colloquim on Cytogenetics of Domestic Animals p. 19.

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LOPEZ-CORRALES, N.L., SONSTEGARD, T.S., et al. 1998. Comparative mapping: conserved physical location of PROC, EN1, ALPI, TNP1, and IL1B genes in cattle, sheep, and goat. Proc. 13th Int. Chromosome Conf., p. 23.

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MISZTAL, I. ... and VAN TASSELL, C.P. 1998. Studies on the value of incorporating effect of dominance ... dairy cattle, beef cattle, and swine. Proc. 6th World Congr. Genet. Appl. Livest. Prod. 25:513-516.

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PHILPOT, J.C., WIGGANS, G.R. and VAN TASSELL, C.P. 1998. Use of the World Wide Web to distribute genetic evaluations. J. Dairy Sci. 81(1):62.

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POWELL, A.M., CLEMENTS, J., ZINK, C., ASHWELL, M.S., WALL, R.J. and REXROAD, JR., C.E. 1998. Transmission and effects of Visna virus transgenes. Theriogenology 49(1):391.

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SMITH, T.P.L., KAMBADUR, R., MRIDULA, S., BASS J.J., CASAS E., STONE R.T., KAPPES, S.M. ... and SONSTEGARD, T.S. 1998. Myostatin mutations cause double muscling in cattle. Plant Animal Genome 6, p. 32.

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SONSTEGARD, T.S., KAPPES, S.M., KEELE, J.W. and SMITH, T.P.L. 1998. Refinement of bovine chromosome 2 linkage map near the mh locus ... between the bovine and human genomes. Anim. Genet. 29(5):341-347.

Publications: (Continued)

19.
SONSTEGARD, T.S., KAPPES, S.M., et al. 1998. Refinement of bovine chromosome 2 linkage map near mh locus ... gene order rearrangements between the bovine and human genomes. Plant Animal Genome 6 p. 155.

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SONSTEGARD, T.S., ROHRER, G.A., and SMITH, T.P.L. 1998. Myostatin Maps to Porcine Chromosome 15 by Linkage and Physical Analyses. Anim. Genet. 29(1):19-22.

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VALLET, J.L., SMITH, T.P.L., SONSTEGARD, T.S. and HEATON, M.P. 1998. Structure of the gene for porcine endometrial folate binding protein. J. Anim. Sci. 76(Suppl 1):240.

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VAN TASSELL, C.P., et al. 1998. Method R estimates of heritability, repeatability, and dominance fraction of variance for milk, fat, and protein yields of Holstein dairy cattle. J. Dairy Sci. 81(1):70.

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ZEGEYE, A., ASHWELL, M.S., REXROAD, JR., C.E. and MATHER, I.H. 1998. Quantitative trait locus (QTL) analysis of the bovine butyrophilin gene. J. Dairy Sci. 81(1):73.

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0402309 Year: 98 Project Number: 1265-31000-061-02 S
Mode Code: 1265-45-00 STP Codes: 3.1.2.1 100%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

Title: CREATION OF A RESOURCE POPULATION OF DAIRY CATTLE

Period Covered From: 09/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

All current investigations of quantitative trait loci (QTL) in dairy cattle populations utilize the granddaughter design". Even though this design is very powerful for detecting QTL, statistical limitations inherent to the population hinder the process of identifying lowly heritable traits across families and the causative genetic variation underlying all traits. Previous QTL investigations in AF-2 design@ beef cattle populations have been successful at finding traits of low heritability (marbling, tenderness, etc.) and refining the genetic intervals near all trait loci, thus simplifying the development of DNA marker tests to assist selection of important economic traits in commercial populations. We (USDA-Beltsville/Univ. of MN) have initiated a project to develop an F-2 Holstein population using MOET to create approximately 864 females derived from founder lines that are phenotypically different for milk yield and somatic cell score. This dairy population will be a powerful resource for analyzing a wide range of trait loci involved with milk production, protein and fat yields, protein and fat percentages, productive life, somatic cell score (an indirect measure of mastitis), calving ease, and conformational type traits.

2. How serious is the problem? Why does it matter?

The goal of livestock QTL research is to improve selection of genetically superior animals through the use of genetic markers in a quick, efficient, and reliable manner. Even though numerous investigations have identified QTL for milk production, most of these

loci are not yet amenable for commercial use in marker-assisted selection of QTL. This is primarily due to statistical limitations of the experimental populations where calculating power is limited for QTL interval confirmation and refinement. Statistical power could be improved by increasing family size, phenotypic differences, and informative genotypes. These experimental attributes are not easily altered with the current available QTL mapping resources, which ultimately ends up deterring the development of reliable QTL selection markers. An F-2 population is a more robust experimental design for detection and location (mapping) of QTL in domesticated animals, as

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shown by studies in beef cattle, pigs and chickens. Because the F-2 design has not been applied to dairy cattle and it is more powerful than the granddaughter design, we expect to identify and confirm: new QTL, QTL caused by non-additive effects, QTL that are fixed in active sire lines, and QTL previously identified in other populations; all within genetic intervals that are more easily refined and developed into markers for selection.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program 101 Animal Germplasm, Resources, Conservation, and Development (100%) This population will be an important resource for scientists to identify DNA markers associated with the genetic variation in genes affecting milk production efficiency, disease resistance, reproductive efficiency, and longevity in dairy cattle. These cattle preserve and allow experimental access to "outdated" genetic material from Holsteins produced before the application of biometrical selection. Once analyzed, this germplasm will serve as a unique resource of Alost@ genetic material critical needed to improve lowly heritable traits in current generations of cattle. For example, research has shown that genetic gains in milk production have produced setbacks in fertility and disease resistance. Some of these traits critical to improving animal longevity were probably squandered by 35 years of selection for milk production.

4. What was your most significant accomplishment this past year?

Eight bulls that will be used in the mating scheme to produce F1 progeny were identified and semen for each was purchased. The first eight cows were treated to induce multiple ovulations, and artificially inseminated. The embryos were recovered from the eight cows and frozen for subsequent transfer into recipient cattle.

5. Describe your major accomplishments over the life of the project,

including their predicted or actual impact

Some of the QTL information generated from this population will be unique with respect to studies conducted on granddaughter design populations; thereby, increasing knowledge of cattle biology and milk production. All the QTL-marker information will be directly applicable to the dairy cattle breeding industry to further improve selection decisions and increase the health, fitness, reproduction, and production of the commercial dairy cow.

6. What do you expect to accomplish during the next year?

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About 25 more cows will be treated to produce about 300 embryos for transfer into recipient cows to produce about 100 calves. As a preparatory step for the genome scan of quantitative trait loci, the expected heterozygosity index of 200 markers will be determined.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

No technologies are currently available for transfer. QTL and associated DNA marker information will not be available for commercial application until the F-2 population is constructed and analyzed on a genome-wide basis.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:

Approved: D.F. COLE Date: 02/99
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Accession: 0149375 Year: 98 Project Number: 1265-31000-066-00 D
Mode Code: 1265-45-00 STP Codes: 3.1.2.1 80% 3.1.3.1 20%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

Title: MOLECULAR AND CELLULAR STRATEGIES TO ENHANCE THE GERMPLASM AND GENOME OF LIVESTOCK

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Traditional genetic selection methods have made extensive contributions and steady progress toward improving animal productivity and product quality. However, the rate of genetic progress in livestock is slow because of long generation intervals, and genetic markers associated with economically important traits and disease resistance are lacking. Certain modifications of an animals genetic composition can only be made by direct manipulation of the genome through gene transfer. This project is directed specifically at using gene transfer 1) to produce a leaner market pig, and 2) to produce sheep that might be resistant to infection by visna virus. An additional goal is to establish embryonic stem cells for farm animals so that sophisticated modifications can be made, such as gene deletions or gene replacements.
Problem 1 is the high fat content of swine. The approach undertaken was to introduce a fusion gene that directs expression of insulin-like growth factor-I (IGF-I) specifically in skeletal muscle with the hypothesized consequence being that elevated IGF-I will stimulate muscle development and reduce deposition of fat in the carcass.
Problem 2 is high susceptibility of sheep to visna virus infection. The approach undertaken was to introduce the gene encoding an envelope protein and the LTR for visna virus with the hypothesized consequence that expression of the envelope protein will block subsequent visna virus infection.
Problem 3 is the lack of embryonic stem cells for farm animals. Inner cell masses as well as individual blastomeres from pre-compact embryos will be isolated and cultured on various feeder layers and in presence of various growth factors to determine effective conditions to maintain embryonic

cells in the undifferentiated state to retain their pluripotency.

2. How serious is the problem? Why does it matter?

In spite of more than forty years of genetic selection for leaner pork the average eviscerated hog carcass contains about 33% fat, much of which is trimmed from the retail product. If carcass lean content were reduced by 20% and carcass lean increased proportionately it would save the swine industry more than $ 1 billion annually in feed and labor that goes into producing lard, which is the lowest priced commodity derived from hog

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NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

carcasses.
Infection of sheep with visna virus is a widespread problem throughout the world. In many locations up to 75% of specific flocks are infected. Most visna virus infections occur in the newborn lamb. The disease is manifest in sheep primarily as progressive pneumonia as a result of depressed immunity in the adult sheep. Total elimination of infected flocks has been the only effective means to control this virus, since immunization has been effective.
While additional copies of genes can be inserted into the genome of farm animals by microinjection into the pronucleus of the early embryo, more sophisticated genome modifications, such as gene deletions or gene replacements, were thought to require the establishment of long term culture of embryonic stem cells or primordial germ cells for each of the livestock species. Such highly specific gene manipulations have thus far been possible only in mice. The cloning of the now famous Dolly in 1997 has done much to change that viewpoint. It now appears that fibroblasts, and possibly other types of cells, might be amenable to genetic manipulations during in vitro culture and then subsequently used for nuclear transfer to produce live progeny. The ability to perform such genetic manipulations in livestock may be extraordinarily important to the future capability to successfully modify animals to impart new production traits, to control diseases, and to produce new animal products.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This research relates to National Program 101, Animal Genomes, Germplasm, Reproduction, and Development 100%. The use of molecular and cellular biology provides new opportunities for rapid genetic progress by manipulating the genomic DNA to control the expression of individual genes and thereby improve productive efficiency of farm animals and possibly produce animals with resistance to viral diseases. Research on embryonic stem cells, primordial germ cells and other mammalian cells maintained by in vitro culture may also lead to advanced techniques in germplasm preservation.

4. What was your most significant accomplishment this past year?

Our most significant accomplishment was establishment in the laboratory of a somatic cell nuclear transfer technique for cloning of cattle. Multiple embryo clones were produced using fibroblast cells that were isolated from a calf fetus, maintained by in vitro culture for several months, and subsequently fused during nuclear transfer to oocytes from which the nuclear material had been removed. After seven days of in vitro culture, several of these cloned embryos were transferred into recipient host cows

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to determine the potential of these clones to maintain pregnancy and develop. The work resulted in the creation of two nuclear cloned Jersey calves that were carried by the host cow for 8-months before being lost as a result of excessive fluid retention that overwhelmed the ability of the cow to carry the pregnancy to full term.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Transgenic pigs were produced that expressed elevated insulin-like growth factor-I (IGF-I) in skeletal muscle. When these growth factor transgenic pigs reached market weight they had 10% less carcass fat and 8% more lean muscle than sibling control pigs. This increased the carcass value by an estimated $6.00 per market hog. In addition, the overall health status of the IGF-I transgenic pigs did not differ from that of sibling control pigs. This finding may be of sufficient interest to swine breeding companies that they may consider the commercial potential of growth factor transgenic pigs in the future.

Visna virus genes for envelop protein (ENV) and transactivator protein (TAT) were transferred into sheep. The resulting transgenic progeny expressed these genes. This expression did not cause pathological problems. However, when these transgenic lambs were injected with an Icelandic strain of visna virus several of the transgenic lambs became infected with the virus in the same manner as the control sheep. This finding provides evidence that expression of viral envelope protein in cells may not be a useful strategy for preventing viral infection. This finding will be extremely useful in guiding other researchers in designing disease prevention strategies.

While our attempts to establish embryonic stem cells for pigs, sheep and cattle have been unsuccessful, our research has never the less produced some very useful information and technology advances. First, a pig liver stem cell line (PICM-19) was isolated and characterized from pig embryonic cells. The PICM-19 cell line is the subject of two patents and may be useful in artificial liver devices and for assay of agents that are toxic to the liver. Second, we developed a simple method for the continuous

culture of embryonic, fetal and adult pig macrophages that may be useful in the study of the relationship between the PRRS virus and the macrophage, which is the target cell for this virus. Third, a bovine trophectoderm cell line (CT-1) was established and demonstrated to produce interferon-tau, which is a marker for this cell type. The production of interferon-tau from CT-1 cells may have potential utility in animal reproduction, anti-viral therapy, and anti-cancer therapy. Forth, a bovine endoderm cell line, CE-2B, was established and demonstrated to produce serum-proteins. The CE-2B and CT-1 cell lines may be useful in nuclear transfer experiments involving homologous recombination since they have a long life span in culture.

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6. What do you expect to accomplish during the next year?

We expect to improve the cloning procedures for cattle by using information learned from systematic evaluation of nuclear transfer protocols and various in vitro culture conditions used for oocytes, donor cells, and embryos up to the time of transfer, and from investigating the developmental capacity of aggregated embryos.
We will isolate and evaluate several epithelial cell lines and test them for there potential for use in nuclear transfer experiments. Specific aims will be to establish several cell lines, demonstrate their extra longevity in culture in comparison to fibroblasts, develop methods for transfection of genes into the cell lines, and evaluate their usefulness as nuclear donors for the production of cloned calves.
We will transfer one or more myostatin antisense transgenes into mice to determine the feasibility of stimulating muscle development by this approach in contrast to removing the myostatin gene, which has previously been done in mice and occurs naturally in some breeds of cattle. If results are favorable the experiment will then be expanded to include gene transfer in swine.
A lysostaphin transgene, which may be effective in reducing the incidence of mastitis in cattle, will be transferred into fetal fibroblasts. The transgenic fibroblasts will subsequently used for nuclear transfer into oocytes to produce cloned transgenic embryos for transfer into host cows to produce transgenic cattle.
A line of transgenic pigs that express the IGF-I growth factor specifically in muscle will be crossed with two lines of hybrid swine to produce transgenic and non-transgenic siblings. The growth rates, feed efficiencies, health status, and carcass composition of these sibling pigs will be compared to determine whether this growth factor may have sufficient commercial potential to the swine industry to warrant further investigation.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer,

other scientists)? What are the constraints, if known, to the adoption durability of the technology?

The CT-1 and CE-2B cell lines were submitted to the cell collection of the Coriell Institute for Medical Research, Camden, NJ, which is a source of materials distributed to researchers on aging. The PICM-19 cell line was submitted to the American Type Culture Collection, Rockville, MD for patent deposit, and the cell line has been licenced to Gene Span Corporation, Redmond, WA and to Cell Technologies, Inc, San Diego, CA. One patent has been issued on the PICM-19 cell line, and a second patent has been allowed

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(awaiting issuance) for the use of the PICM-19 cell line in artificial liver devices. The constraints to the adoption and durability of the technology are not known.
The growth factor transgenic pigs were produced under a CRADA with GeneMedicine Inc., The Woodlands, TX. The existing pigs will not be commercialized because a human growth factor gene was used for the experiment, therefore, market of such animals would be unacceptable and inflammatory to consumers. If a growth factor gene from a farm animal species was used we do not yet know whether such transgenic animals would be accepted by the public. Regulatory agency approval (USDA, FDA) would also be required. Information about the progress with the growth factor transgenic pigs was presented to the National Pork Producer Council at a workshop held at the National Swine Research Center, Ames Iowa.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

TALBOT, N.C. and CAPERNA, T.J. 1998. Selective and organotypic culture of intra hepatic bile duct cells from adult pig liver. In Vitro Cell. Dev. Biol. 34: 785-798.
N.C. Talbot presented a Keynote Address entitled "The PICM-19 cell line: an in vitro model of pig fetal liver stem cells"on December 10-11, 1998 at a Hepatic Stem Cell Meeting, held at the National Institutes of Health, Bethesda, MD.
V.G. Pursel presented an invited paper entitled "Expression of IGF-I in skeletal muscle of transgenic swine" at the joint meeting of American Dairy Science Association and American Society of Animal Science held in Denver, Colorado.

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MITCHELL, A.D., SCHOLTZ, A.M., PURSEL, V.G. and CLOVER, C.M. 1998. Composition analysis of pork carcasses by dual-energy x-ray absorptiometry. J. Anim. Sci. 76:2104-2114.

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TALBOT, N.C. and CAPERNA, T.J. 1998. Selective and organotypic culture of intra hepatic bile duct cells from adult pig liver. In Vitro Cell. Dev. Biol. 34:785-798.

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Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400806 Year: 98 Project Number: 1265-31000-067-00 D
Mode Code: 1265-45-00 STP Codes: 3.1.1.3 40% 3.1.2.1 60%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

Title: IDENTIFICATION AND MANIPULATION OF GENETIC FACTORS TO ENHANCE MILK PRODUCTION AND QUALITY

Period Covered From: 10/96 To: 09/01

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Two major constraints on dairy productivity and efficiency are: (1) limited persistency of lactation and (2) mastitis. Normal management of dairy cows provides for a lactation of approximately 300 days and a 60 day dry period. This accommodates the average length of a dairy cow's lactation, while providing for a dry period of ample length to maximize production in the next lactation. However, if the length of a productive lactation could be increased (i.e., increased persistency), difficulties associated with breeding lactating cows would be reduced and lifetime milk yield and production efficiency would be increased. Mastitis is the most costly disease of dairy cows because of its negative impact on milk revenue, milk quality and increased veterinary costs. Enhancing a cow's resistance to mastitis pathogens would provide a financial benefit to the dairy industry and a health benefit to the consumer. A third area that is not a problem, but rather is an opportunity, is utilizing new technology to create new and improved animal products such as milk that can be processed more efficiently into cheese and milk that contains value-added nutritional properties. This CRIS will address all three of these key issues - increasing persistency of lactation, enhancing natural defense against mastitis and creating new milk products. The genes regulating cell proliferation and programmed cell death are being investigated. Once identified, the control of these genes will be altered to increase the duration of lactation. These investigations are being pursued using both mouse models and dairy cows. Both endogenous genes such as lactoferrin and genes from other species such as lysostatin are being investigated to enhance mastitis resistance of dairy cows. Under the control of a

genetic switch which is currently being evaluated in mice, and utilizing cloning techniques being developed by others in the lab, these genes will be used to produce transgenic cattle. Once genes are identified that control mammary cell turnover, transgenic animals will be produced to test our ability to extend the duration of lactation.

To address these problems we will:

a) Quantify cell turnover during lactation; b) determine the underlying basis for the decline in milk yield during lactation; c) characterize the population of mammary epithelial stem cells; d) develop a mammary gland specific gene expression cassette; e) develop a reliable method

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for evaluating the influence of milk modification strategies; f) increase cheese yield by increasing casein content of milk; g) increase resistance to mastitis by introducing antibacterial genes into mammary glands.

2. How serious is the problem? Why does it matter?

Progress in the areas of investigation that are identified in this CRIS will have important benefits to the dairy industry.
Lengthening the duration of lactation would increase a cow's lifetime milk production, milk production efficiency, and would also reduce management constraints imposed by the necessity to successfully rebreed a cow within three months of parturition. Achieving this goal would be a major advance and would produce profound effects on the dairy industry. The financial benefit of simply eliminating the negative impact of the number of days open can be estimated at 300 million dollars annually; however, other financial benefits also will accrue.
Mastitis is the most costly disease of dairy cows and has hidden health dangers for the consumer. The combined impact of lost milk revenue and increased veterinary costs due to mastitis has been estimated to be in excess of 2 billion dollars annually. Increasing the mastitis resistance of dairy cows would lessen this financial drain. Additionally, it would provide the added public health benefit of reducing antibiotic therapy, with its inherent risks of food contamination, allergic reaction for the consumer, and increased risk of bacterial resistance to antibiotics.
The impact of enhancing milk processing attributes and nutritional value will similarly have major impact on the dairy industry. For example, the financial benefit of increasing the casein concentration of milk by 12% would increase the profitability of making cheese by approximately $23 million in average size cheese processing plant.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This CRIS falls under the Animal Germplasm, Resources, Conservation and Development National Program and within the Animal Genome National Program Component.
Defining the physiological mechanisms that increase lactational efficiency and persistency should not only lead to identifying desirable alleles but also provide the knowledge base needed to manipulate that trait for benefit of the dairy industry.
Developing the tools for controlling gene expression in the mammary gland will allow the research community to take advantage of current knowledge and that which will emerge from genome mapping efforts to

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introduce genetic changes in the shortest period of time and in a highly cost effective manner. Furthermore, the genetic engineering tools will provide a means of improving mammary gland function in ways that can not be achieved by selective breeding.

4. What was your most significant accomplishment this past year?

The animal bioreactor industry was founded on the premise that pharmaceuticals could be produced in the milk of transgenic farm animals in a cost effective manner. As the industry matures it has come to realize that purification of drugs from milk is their most significant production cost. In an effort to demonstrate the feasibility of reducing those costs by producing pharmaceuticals in a fluid less complex than milk, transgenic mice were produced carrying a bladder specific regulatory element driving the coding region for human growth hormone. As hoped, human growth hormone was secreted into the urine and the level of production was more or less constant over the 8 months of sampling. We demonstrated that unlike the mammary gland bioreactor system, one can harvest product from both sexes within days or weeks of the animals birth. This accomplishment is too new to have a measurable impact. However, Nature Biotechnology felt the paper noteworthy enough to be published with an accompanying commentary. The project also engendered an enormous amount of press attention. Outlets as diverse as the British Medical Journal and Esquire requested interviews. TV news segments appeared in Brazil, England, Japan, US and elsewhere. Scientific American and the Smithsonian Magazine reported our results. Students from at least 3 universities used the paper as the focus of class projects. It is too soon to tell if the bioreactor industry will adopt this strategy, though several invitations to discuss this work at industry conferences have been forthcoming.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Lacking a clear understanding of how regulatory elements control spatial

and temporal gene expression compromises our ability to elucidate molecular mechanism of development and to modify animal traits. To overcome this difficulty, we are developing a synthetic genetic switch that is composed of sequences from five different organisms. The switch was used to control expression of SV40 T antigen, a known viral oncogene. As expected, when the switch was turned on, hyperplasia was observed in submandibular salivary gland. Turning the switch off at 4 months of age reversed the hyperplasia, however, turning the switch off at 7 months did not alter turmorigenesis. This is the first report of a phenotype being controlled by a synthetic genetic switch.

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6. What do you expect to accomplish during the next year?

By using markers for cell proliferation and applying morphological criteria established for putative stem cells in mouse mammary gland, we will attempt to identify stem cells from mammary tissue of young calves and to establish these cells in vitro. If successful, these cells will provide a valuable source for in vitro investigation of mammary cell physiology and for the genetic manipulation of lactational parameters. Current investigations of genetic regulation of cell turnover will continue. In particular the role of p53 in mammary cell turnover and function will be investigated in knockout mice and expression of genes involved in mammary cell growth and apoptosis will evaluated during lactation of dairy cows. Finally, a bovine mammary expression library will be produced to provide a resource for identifying genes that are differentially regulated with physiological state.
Refinement of our genetic switch will continue. Modifications will be made to decrease "leaky" expression and to increase the amount of product produced when the switch is turned on. These modifications will be tested in tissue culture and the genetic alterations found to enhance the performance of the switch will be used to build a mammary gland specific switch to drive the expression of alpha-S1-casein, the predominant milk protein. The switch-controlled alpha-S1-casein gene will be introduced into mice. The resulting transgenic mice will be evaluated to determine the effect of increasing the amount of protein in milk on the function of the native mouse milk proteins.
A mammary gland specific gene that encodes the lysostaphin protein, which specifically kills S. aureus, will be introduced into mice. S. aureus is a major cause of mastitis in dairy cows. Mastitis caused by Staphlococcus aureus is highly transmissible and difficult to cure..
The resulting transgenic mice will be inoculated with S. aureus to determine the efficacy of lysostaphin to block and or reduce the severity of mastitis.

7. What technologies have been transferred and to whom? When is the

technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

What technologies have been transferred and to whom? When is the technology likely to become available to the end user? What are constraints, if known, to the adoption & durability of the technology? Our technology was shared with scientists through invited presentations at two international meetings in Korea (8th World Conference on Animal Production, Seoul, Korea, July 2, 1998.) and Japan (Japan Society for

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the Promotion of Science annual meeting on Molecular Bioengineering of Food Animal Protein Resources, Tokyo, Japan, October 23, 1998) and at one national meeting in the US (American Society of Animal Science American Society of Dairy Science joint meeting, 1998).
Our technology was shared with the general public through press interviews with UPI, London Daily Telegram, Toronto Globe & Mail, New Scientists (London), Reuters, Biotech World, The Why Files (Internet science website), Science News, Biofutur (a French magazine), British Medical Journal, Scripts (Pharmaceutical industry trade journal), Idea Television (Brazilian public television), ScienceNow (Science's website news feature), BBC, Molecular Medicine Today (London), and Scientific American, National Public Radio (All Things Considered and Morning Edition), ABC News (radio interview), WNYC Insight (radio talk show), Genetic Engineering News, and Baltimore Sun.
We discussed the implications of genetic engineering and cloning technology on network evening television new programs (CBS, NBC and CNN). Featured articles discussing our research appeared in Smithsonian Magazine, Scientific American, Kiplinger Agricultural News Letter, Nature Biotechnology, Washington Post, LA Times, Chicago Tribune, Yahoo News, Chuck Sheperd's nationally syndicated column, and others.
Invited by Nature to participate in a news conference in New York on the announcement of the first mouse clones entitled "Dolly the sheep, Mickey the mouse: Experts discuss implications of recent advances in mammalian cloning."
The underlying technology is already being utilized by the Biopharmaceutical industry with success. Further development of the technology will be required before it is appropriate for adoption by the animal production industry.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

KERR, D.E. , BONDIOLI, K.R., ZHANO, H., LIANG, F., WALL, R.J. and SUN, T-T. 1998. The bladder as a bioreactor: Urothelium production and

secretion of growth hormone into urine. Nature Biotechnology 16:75-79.
Smithsonian Magazine, July 1998. New Breeds Down on the Pharm. By Albert Rosenfeld. Pages 22-30.
CAPUCO, A.V. and J. BYATT. 1998. Cell turnover in the mammary gland. Symposium on Cell Turnover in Repro. Processes at the joint national meeting of the Am Dairy Sci Assoc and the Am Soc Animal Sci.

PUBLICATIONS:

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WALL, R.J. 1997. Transgenic Livestock: Progress and Future Prospects. pp. 9-18. IN: N. Li and Y. Chen (eds.) Proc. of International Conference Animal Biotechnology. International Academic Publishers, Beijing.

Publications: (Continued)

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Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

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